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**PAPER**

# Detection and quantification of traces of bisphenol A and bisphenol S in paper samples using analytical pyrolysis-GC/MS

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In this paper a simplified method based on analytical pyrolysis gas chromatography mass spectrometry (Py-GC/MS) for the detection and quantification of bisphenol A and bisphenol S in paper samples is presented. The method enables a direct analysis of the samples without tedious sample preparation. As the analytes are thermally desorbed, a solvent extraction is not needed. The method is applicable to small samples of ~120 μg. The limits of detection are below 1 mg $kg^{-1}$ for bisphenol A and for bisphenol S. The limits of quantification are about 1.3 mg $kg^{-1}$. Several validation characteristics of the method developed like standard error of calibration, limit of determination, linearity, and accuracy are given. To prove the accuracy of the method, interferences and matrix dependencies were also investigated. The influence of the pyrolysis crucibles, a special effect of analytical pyrolysis, was additionally investigated. It was found that the impact of the crucibles on the results is significant and one cause for matrix effects.

## 1. Introduction

Paper is an extensively used versatile product. It has become an integral part of our daily life. Its fields of application are office paper, tissue, packaging, decoration, newspapers, journals, and more. As frequent contact with paper is inevitable, natural concerns exist about the content of compounds that might affect human health. 4,4′-Dihydroxy-2,2′-diphenylpropane, usually known as bisphenol A (or BPA) is one of these compounds, whose content in paper is important to evaluate for reasons related to health and the environment. Bisphenol A is used as a significant component in the production of epoxy resins and polycarbonates. In its monomeric form, it is used as an additive in the production of thermal printer paper and carbonless papers.[1] The production of bisphenol A amounted to 1.15 million tons in Western Europe during 2005–2006. Of this amount about 0.2% is used for the production of thermal paper.[2] In 2010, the European Thermal Paper Association (ETPA) reports that bisphenol A is currently used mainly for point-of-sale (POS) receipts (*e.g.* supermarket bill receipts), but also for self-adhesive labels, lottery tickets, and fax paper.[2]

The effects of bisphenol A have been widely investigated in bioassays as xenoestrogen, a compound that mimics the behaviour of estrogens. In 1939, the first study on bisphenol A attempted to synthesize compounds that mimic female's hormone behaviour.[3] In spite of this fact, bisphenol A is unsuitable for medical application because of the lower estrogenic activity of bisphenol A compared with newer developments.[4] Some years later, bisphenol A was found apt for the production of polycarbonates and other applications. During the last few decades, discussion about the convenience of bisphenol A has arisen concerning the potential impacts on human health.[5–8] The studies about BPA are not conclusive.[9–15] The controversy about the impact of bisphenol A on human health includes the implications of different metabolisms of the studied species, the reliability of the methods used in these tests, and the conflicts of interests of some laboratories.[16–21]

The controversy over bisphenol A has involved various non-governmental and governmental assessment bodies.[19] In 2003 an extensive risk assessment report about BPA, developed by the Institute for Health and Consumer Protection (former European Chemicals Bureau), found no risks for consumers.[22] These results were confirmed in 2008.[23] In 2006 the European Food Safety Authority (EFSA) established a TDI (Tolerable Daily Intake per person) of 0.05 mg $kg^{-1}$.[24] This figure was confirmed in 2010.[25] In 2008 EFSA reported that the exposure of fetuses to bisphenol A is negligible and that the bisphenol A released from materials in contact with food does not affect human health.[23] However, other institutions have established more strict regulations on BPA. In Canada, BPA was found hazardous to human health in 2008.[26] Canada was the first country to ban bisphenol A in baby bottles.[27] In North America, the U.S. Food and Drug Administration (FDA) has raised concerns about exposure of fetuses, infants, and young children to BPA.[28] The U.S. Environmental Protection Agency (EPA) has declared BPA a chemical of concern and implemented an action plan to remove it from industry.[29] In Europe, the European Commission declared a ban on BPA for the manufacture of baby bottles from June 2011.[30]

Human exposure to bisphenol A is significant.[31,32] In some regions, high levels of bisphenol A have been detected in air, water, and dust.[33,34] Some paper products are sources of BPA.[35]

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In North America, thermal paper in receipts contains relatively high amounts of BPA from 0.3 to 1.7%.[36] The ETPA estimates that around 30% of thermal paper used enters in recycling streams.[22] Though most impurities of paper are removed during cleaning in the recycling process, some impurities remain in the final product. In Japan, BPA was found in 67% of the investigated food contact papers containing recycled fibers amounting to 0.19–26 mg $kg^{-1}$.[35] Products from recycled paper like kitchen paper rolls contain about 0.3–4.7 mg $kg^{-1}$ (Ref. 37) and toilet paper 3.2–45.5 mg $kg^{-1}$.[38]

Labelling organisations, which are members of the Global Eco Labelling Network,[39] attempt to regulate the quality of recycled paper: the "Environmental Choice" in New Zealand, the "Green Seal" in USA, and the "Federal Environmental Agency" in Germany. To obtain the ecolabelling granted by the "Federal Environmental Agency" called "der Blaue Engel", the quantification of BPA is required, though limits for the amount of BPA in paper have not yet been established.[40] In order to achieve the basic criteria for the environmental label, the "Blaue Engel", a quantification method for BPA has been established in the RAL-UZ14 (EN 645).[40] This methodology was originally developed by the European Committee for Standardization ECN (CEN-TS13130-13) for utensils in contact with food.[41] After the extraction of BPA from paper samples with cold water, the water extract is filtered and separated *via* HPLC. BPA is detected with UV or fluorescence rays. This method detects the amount of leached BPA.

The identification and quantification of bisphenol A is a frequently studied issue.[42–44] However, there is little documentation about the quantification in paper samples. Vinggaard *et al.*[37] extracted bisphenol A from kitchen paper rolls by means of soxhlet extraction with ethanol. The solvent was evaporated; the extract was methylated, an internal standard applied, and finally analyzed *via* GC/MS. Gehring *et al.*[38] extracted the BPA with a soxhlet apparatus with cyclohexane–ethyl acetate 98.5 + 100 (v/v) as a solvent for over 10 h and cleaned up the extracts by means of size exclusion chromatography (SEC). Derivatisation was carried out in a silica gel bed and the extract was analyzed with GC/MS. Ozaki *et al.*[35] refluxed paper samples with ethanol for 2 h and carried out a second extraction with DCM. The solvents were removed by evaporation and the residues dissolved in *n*-hexane. BSTFA was applied as a silylation agent before the analysis with GC/MS. Mendum *et al.*[36] quantified the content of bisphenol A in thermal paper receipts by extraction with ethanol at 35 °C, followed by derivatisation with TMSI, and quantified *via* GC/FID. All these techniques include an extraction step that is a source of analysis errors. Furthermore, extraction is time and solvent consuming and, therefore, expensive.

Bisphenol S is an equivalent material to bisphenol A. It has two phenol functional groups on either side of a sulfonyl group. Instead of bisphenol A, bisphenol S is commonly used in the production of epoxy resins and as an additive in thermal paper. Bisphenol S has not been as widely investigated as bisphenol A has. Some assays about the impact of bisphenol S on human health report weaker estrogenic activity.[45,46]

This article describes the development and validation of a new method for the quantification of impurities like bisphenol A and bisphenol S in paper. The method for the quantification of bisphenol A and bisphenol S proposed here is a direct method that combines analytical pyrolysis with gas chromatographic separation and mass selective detection (Py-GC/MS). This technique is adequate for the quantification of additives in solid samples. Other methods for additive quantification in solid samples have been developed in other works.[47,48] Former studies do not present proper validation of the methods proposed. In those studies, information about limits of detection as well as the accuracy of the methods is lacking. This study aims at quantifying paper impurities or trace elements[49] in paper samples at a low concentration (~1 mg $kg^{-1}$), by giving meaningful key values for the quality assessment of the proposed method. The quantified samples allow the validation of the method and are not used to report the average concentration of BPA or BPS in paper products.

## 2. Materials and methods

### 2.1. Materials and reagents

Bisphenol A (99% purity grade) and bisphenol S (98% purity grade) were acquired from Sigma Aldrich as well as tetramethylammoniumhydroxide (25 wt% in $H_2O$). Acetone, HPLC grade, was obtained from Merck (Darmstadt, Germany).

Various stock solutions of bisphenol A and bisphenol S were prepared with acetone as the solvent with different concentrations. Diluted solutions are required for the preparation of the calibration samples and were prepared from the previous stock solutions. Each diluted solution was made from a different stock solution; similarly, each calibration level was prepared with a different diluted solution in order to prevent propagating errors.

Hand sheets without any additives were produced from industrially produced fully bleached spruce acid sulfite pulp (SSP) in our laboratory according to Zellcheming standard V/8/76 and served as a reference sample for the calibration samples. The SSP serves as a blank substrate free of any analytes (BPA and BPS).

Six industrially produced thermal papers, labelled as BPA-free, two samples of recycled letter paper, and one sheet copy paper with virgin fibers were used for the validation of the method. For the validation, samples with different concentrations of BPA were preferably selected.

For the test of interferences in samples without any BPA or BPS, handmade sheet paper produced in our laboratory from bleached eucalyptus pulp and office copy papers made from virgin fibers received from two different producers were used.

### 2.2. Pyrolysis-GC/MS

Py-GC/MS analysis was carried out in a Frontier Lab Micro furnace Double-Shot Pyrolyzer (Py-2020iD) equipped with an Autosampler (AS-1020 E). The temperature for pyrolysis was set at 500 °C and the temperature in the interface at 360 °C with a pyrolysis time of 0.5 s. Helium was used as the carrier gas for pyrolysis and gas chromatographic separation. After pyrolysis, the pyrolysate was directly transferred into a DB5 fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) with a split ratio of 30 : 1.

For gas chromatographic separation the flow rate was 1 ml $min^{-1}$. The oven program of the gas chromatograph started

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immediately at 100 °C, with increments of 10 °C $min^{-1}$ up to 250 °C. Once the temperature reached 250 °C, the heating rate changed to 5 °C $min^{-1}$ until 275 °C. Once the temperature reached 275 °C the heating rate changed to 15 °C $min^{-1}$ until 320 °C. This temperature was kept constant for 5 min. For mass spectral detection Agilent 5973N MSD with an electron impact ionisation energy of 70 eV was used. The transfer temperature from the GC to MS was set at 280 °C. The ionisation source and quadrupole temperature were set at 230 °C and 150 °C, respectively. The scan range for the measurement in TIC modus was 35–400 *m/z*. The selective ion mode (SIM) of the mass spectrometer was applied for the quantification of the bisphenol A and bisphenol S with two mass fragments (*m/z* 256 and 241 for BPA, and *m/z* 278 and 123 for BPS).

### 2.3. Sample preparation

Before any preparation, all samples were kept in an isolated room for at least 12 h in order to obtain stable conditions for temperature and humidity. Small sample pieces of 120 ± 5 µg each were cut and put directly into pyrolysis crucibles. The non-analysed samples were used for the determination of the dry solid content. The dry solid content was calculated by weighing the samples before and after air-drying in an oven at 105 °C for 24 h.

In the preparation of the samples used for calibration and in order to achieve the desired concentrations of analytes in paper (from approx. 1 to 100 mg $kg^{-1}$), a volume of approximately 20 µl of the diluted solutions containing bisphenol A and bisphenol S was added to the crucibles. Each crucible contained a piece of the reference substrate SSP of about 120 µg. The volume applied might have varied, depending on the concentration of the analyte in the diluted solution. Each calibration sample was prepared separately from different diluted solutions. Before quantification, new calibration samples were prepared.

After the addition of the derivatisation agent trimethyl ammonium hydroxide TMAH (4 µl of 10 wt% in water) to the crucibles containing calibration and quantification samples, the crucibles were stored for one night with phosphorous pentoxide in a desiccator to remove remaining water. The time between sample preparation and analysis was kept as short as possible. The time span between the preparation and the analyses *via* Py-GC/MS was between 12 and 24 h (see Fig. 1).

### 2.4. Calibration and quantification

Before each analysis, an internal system check (air and water check) was carried out, thereby testing the presence of possible leaks in the system and the sensitivity of the mass spectrometer, with the options provided by Agilent's Chemstation software. For this purpose, mass fragments of *m/z* 18, *m/z* 28, and *m/z* 69 for water, nitrogen, and perfluorotributylamine (PFTBA) were measured. The value of the signal for the corresponding mass fragment of PFTBA *m/z* 69 was used as reference for the sensitivity check of the mass spectrometer. When this value differed from the predefined value by more than 20%, the system was internally calibrated or "auto-tuned" with the three mass fragments corresponding to PFTBA *m/z* 69, 219, and 502 to obtain a similar signal response.

The concentration of bisphenol A and bisphenol S in the paper samples was determined with external calibration. Calibration was repeated before and after each analysis to check possible sensitivity losses in the detector during the measurement of the samples. In addition, for the validation of the method, the concentration of the samples was determined by standard addition.

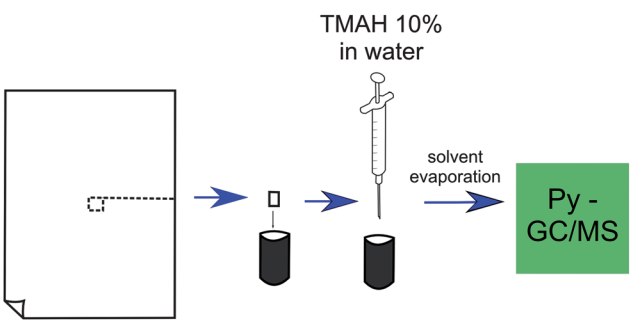


**Fig. 1** Sample preparation.

## 3. Results

### 3.1. Interferences

Direct analytical methods like analytical pyrolysis allow the analysis of samples without—or with few—preparation steps, *e.g.* extraction, separation, and concentration. With this technique, the whole sample is analyzed, not only the analytes of interest, but the paper matrix and other additives as well. This can bring about disadvantages in the form of interferences and matrix effects that may decrease the accuracy of the method.

During the pyrolysis of the analytes containing paper, several analyte specific products are acquired that are separated in the gas chromatograph. Due to the application of TMAH, the methyl ether of the analytes is detected after pyrolysis (shown in Fig. 2). Moreover, the non-derivative form—the original form—of the compounds was not detected. That is a strong indication for a complete reaction of the derivatisation agent

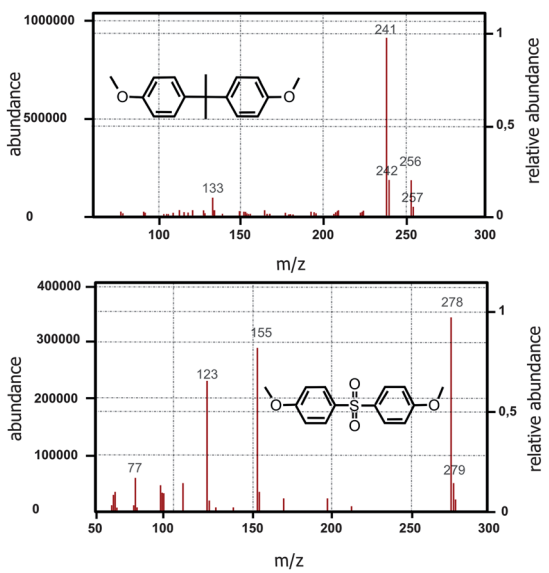


**Fig. 2** Derivative forms of bisphenol A (retention time 13.6 min) and bisphenol S (retention time 18.0 min) detected by mass spectrometry.


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TMAH with both analytes, BPA and BPS. Therefore, we assume that either the complete derivatisation of the compounds occurs or the non-derivative fraction is negligible. The reaction of TMAH and cured epoxy resins based on BPA or polycarbonate would probably give the same products as in the reaction of free bisphenol A with TMAH. Therefore, distinction between free BPA or BPA as polymer constituent cannot be done without further analytical efforts.

The measurement of two mass fragments improves the accuracy of compound identification, reducing method sensitivity to some extent. The measurement of only one mass fragment gives no further criteria to recognize whether the eluting compound is the expected analyte (in this case the methyl ether of BPA or the methyl ether of BPS). Co-eluting compounds with similar retention time may cause overlapping. The identification of the analyte only by its retention time is not reliable enough because of retention time fluctuations due to column performance alteration with time.

For the analysis of the methyl ether of the phenolic compounds, two characteristic mass fragments, *m*/*z* 241 and *m*/*z* 256 for mBPA (methyl ether of BPA) and *m*/*z* 278 and *m*/*z* 123 for mBPS (methyl ether of BPS), were chosen (Fig. 2). The first mass fragment was used for quantification and the second for confirmation. In both cases, the molecular ion was included for an accurate identification of the analyte,[50] for mBPA *m*/*z* 256 and for mBPS *m*/*z* 278.

Close to the retention time of the analyte, very small peaks originating from background interferences, or traces of other matrix compounds may be eluted. These small peaks are lower than the peak area of the compound at the limit of detection for mBPA and mBPS. Despite the fact that the areas are small, these signals can hinder identification at low analyte concentration.

**3.1.1. Interference in analyte determination.** The compounds are identified by determining the ratio between the integrated area of two mass fragments *m*/*z* 256 and *m*/*z* 241 for the methylated bisphenol A and *m*/*z* 278 and *m*/*z* 123 for the methylated bisphenol S. When the ratios differ from the original ratio calculated from the measurement of the pure compounds, this indicates interferences in the identification and, probably, in the quantification. Thus, this means that in such samples the analyte cannot be determined, either because the sample does not contain the analyte or because signals of other co-eluting compounds overlap. In both cases, further analyses with modified parameters are needed.

The pyrograms of paper samples without the analytes bisphenol A and bisphenol S might include signals with small peaks near or at the expected retention times. The integration of these peaks can lead to a wrong analyte identification resulting in false positives. The calculation of the previously mentioned ratios helps to identify the analyte. According to our calculations, the ratio for the identification of the derivative form of BPA is 4.9 and that for the derivative form of BPS is 1.3.

The ratios previously calculated are shown in Table 1. The integrated areas are from surrounding peaks originated from the background. The calculated ratios of these samples differ from the ratios previously calculated for the identification of bisphenol A or bisphenol S. This indicates the absence of both analytes. The different ratios found in the different matrices are, as described above, probably due to matrix interferences; and the integrated area does not correspond to bisphenol A or bisphenol S. The peak areas resulting from samples that do not contain BPA or BPS are lower than the area of the calibration blank (peaks present in our reference substrate SSP do not interfere with the quantification). In addition, the intercept of the calibration plot was always above the value for the calibration blank.

**Table 1** Ratio between measured mass fragments

| | Area *m*/*z* 241 | Ratio *m*/*z* 241 : 256 | Area *m*/*z* 278 | Ratio *m*/*z* 278 : 123 |
|---|---|---|---|---|
| Spruce pulp | 131 034 | 1.4 | 80 982 | 0.9 |
| Eucalyptus pulp | 69 226 | 9.9 | 66 400 | 0.6 |
| Copy paper a | 78 757 | 2.5 | 79 263 | 0.3 |
| Copy paper b | 33 758 | 1.8 | 12 715 | 0.3 |
| BPA/BPS *ca.* 1 mg kg$^{-1}$ | 508 045 | 4.9[a] | 98 566 | 1.3[b] |

[a] Identification ratio for bisphenol A (*m*/*z* 241 : *m*/*z* 256).
[b] Identification ratio for bisphenol S (*m*/*z* 278 : *m*/*z* 123).

The value for the ratio for mBPA and mBPS can be confirmed at each calibration level. This should not vary more than +20% for the accurate determination of the analyte.[50]

**3.1.2. Interference in the calibration.** The matrix type used for the production of the calibration samples might have an effect on the regression parameters that compose the calibration plot. To test matrix interferences, the signal response was evaluated in relation to the analyte content in our paper of reference SSP and in an industrial paper. The SSP handmade sheets do not contain any sizing agents, filler, binder, or other additives contrary to the industrial paper. The paper produced industrially contains a mix of large and short fibers of eucalyptus pulp, and includes sizing agents, mineral fillers, as well as synthetic polymeric compounds as a binder. The industrial paper is covered with a coating layer with an approximate weight of 10 g m$^{-2}$. It does not contain BPA or BPS.

In addition to the investigation on the impact of the paper substrate, the effects of the crucibles were also evaluated. The crucible is the only tool in contact with the samples during the pyrolysis; the crucibles used in our study are made of stainless steel and are covered with a layer of fused silica.[51] This layer is applied to passivate the crucible's surface and hence, to hinder metal contact with the sample. High temperatures and frequent cleaning of the crucibles contribute to a stepwise removal of the coating layer. The loss of the fused silica layer is indicated by the color changes of the crucible surface observed—from light yellow to grey. After layer removal, the contact between the sample and the stainless steel is facilitated. The crucibles, from which the layer was removed, are called "old" crucibles.

The calibration curves that depend on the paper substrate and the quality of the crucibles are presented in Fig. 3. The intercept of all curves does not differ significantly at a statistical level of $\alpha = 5\%$. The linear slopes of the calibration curves on both the spruce paper and the industrial paper do not differ at a significant level of $\alpha = 5\%$. Therefore, the results show no difference with respect to spruce (softwood pulp) or industrial paper (complex matrix) as a matrix material. In contrast, the use of new crucibles changes the slopes of the calibration plot significantly.

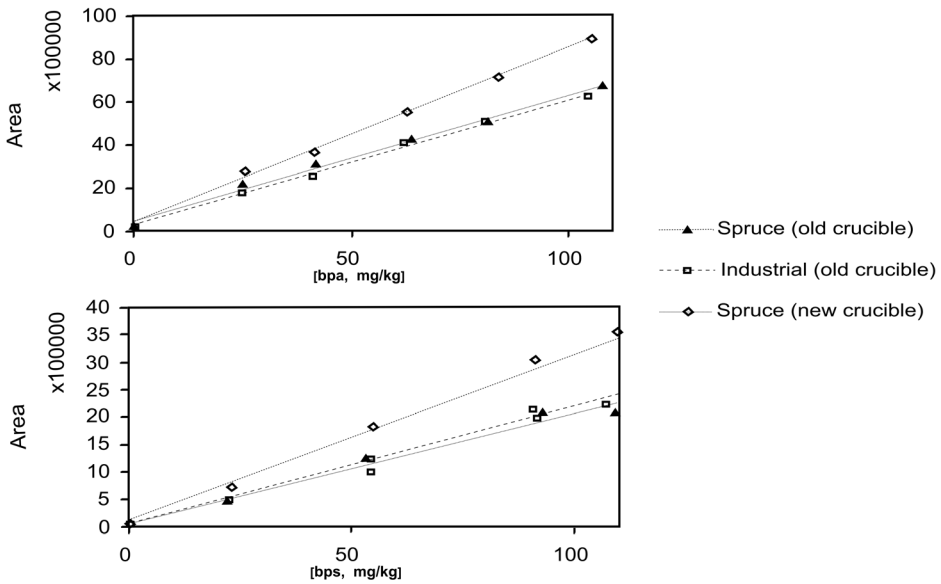


**Fig. 3** Impact of the matrix used on the calibration plot.

The type of crucible used affects the response signal. For the new ones the signal is higher at the same concentration level. This effect is particularly apparent at higher concentration levels. In other words, the signal decreases when old crucibles are used, generating rotational matrix effects.[52]

The influence of the condition of the crucible on Py-GC/MS results has been reported before.[51,53] In these investigations, the quality of the crucible—age—affected the reproducibility of the signal. The reproducibility of the signal was enhanced by using "old" crucibles.

The thermal stability of bisphenol A and bisphenol S at a pyrolysis temperature of 500 °C is sufficient to undergo thermal desorption. Once the crucible enters the hot pyrolysis oven, the analytes bisphenol A and bisphenol S are immediately thermally desorbed from the matrix. The pyrolysates are flushed out of the pyrolysis oven with helium onto the column. The thermal desorption of the analytes from the sample presumably takes place very fast. The analytes are quickly flushed from the pyrolysis oven, before they endure 500 °C. The exposure of the analytes to high temperatures over a longer period would surely lead to the thermal breakdown of the BPA and BPS molecules.

There are several possible explanations for the influence of the crucible. This may be explained by changes in the thermal conductivity induced by repeated cleaning cycles, which may give rise to altered heating rates of the samples. The heat transfer in the old crucibles seems to be more favourable for the thermal desorption of the analytes than in the "new" ones. The "new" crucible restrains the heat transfer from the pyrolysis oven to the sample. In contrast, in an "old" crucible, whose protective layer is worn, the heat transfer occurs faster, and the analytes' exposure to this temperature is longer.

The impact of the surface may not be ruled out either, especially since the TMAH is directly applied as a solution resting as a coating layer on the surface of the crucibles after the solvent has been removed. Since the cleavage of bisphenol A can be enhanced by using a catalyst,[54] the catalyst behaviour of the non-protected surface may also be a reason for the lower signal.

Another possible reason for the weak signal is the incomplete desorption of the analyte, due to the higher porosity of the surface in older crucibles. This possibility was examined but rejected. After analysing the same sample twice under identical conditions, neither BPA nor BPS was detected in the chromatogram of the second run.

### 3.2. Linearity and working range

The limits of determination and the maximum expected concentration of the analyte define the working range for the quantification method. Based on previous analyses[35,37,38] concentrations of no more than 60 mg $kg^{-1}$ are expected. Working with the standard addition method affords adding analyte to the original sample. The concentrations measured in the samples are higher than in the original sample. Hence, the working range selected is from 0 to 100 mg $kg^{-1}$. The decision was taken to extend the upper limit. The estimation of regression parameters and the evaluation of the errors are related to the correlation between signal intensity and analyte concentrations.[55] In addition, the standard addition method requires linearity in the working range.[52]

For evaluating the linearity in the working range, six calibration levels and a blank were used. Each calibration level was prepared twice and separately.

The evaluation of linearity can be carried out by a visual inspection of the calibration plots (Fig. 4), and confirmed by the correlation coefficient values. The correlation coefficients do not improve when second order dependence is assumed. This indicates that a linear relationship is adequate. Additionally, the plot of standard residuals (Fig. 5) was examined according to the recommendations of the Analytical Methods Committee (AMC)[56] for the determination of linearity. The standardized residuals are calculated as the difference between observed and

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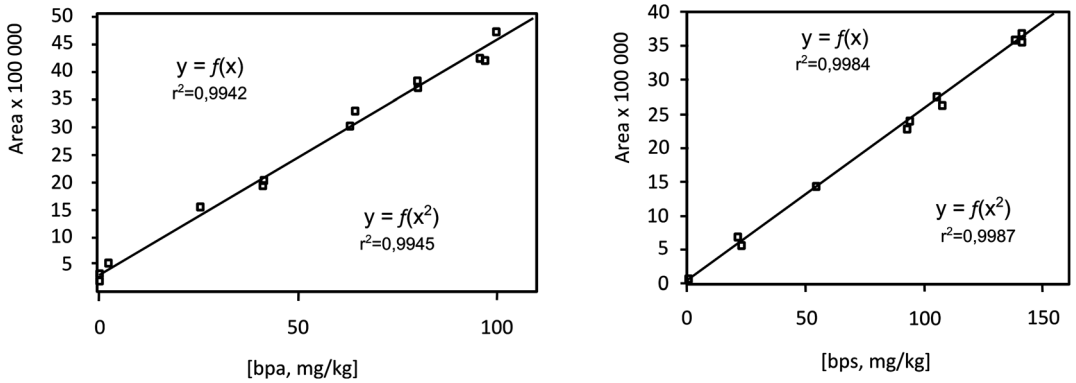


**Fig. 4** Linear and working range of bisphenol A and bisphenol S calibration.

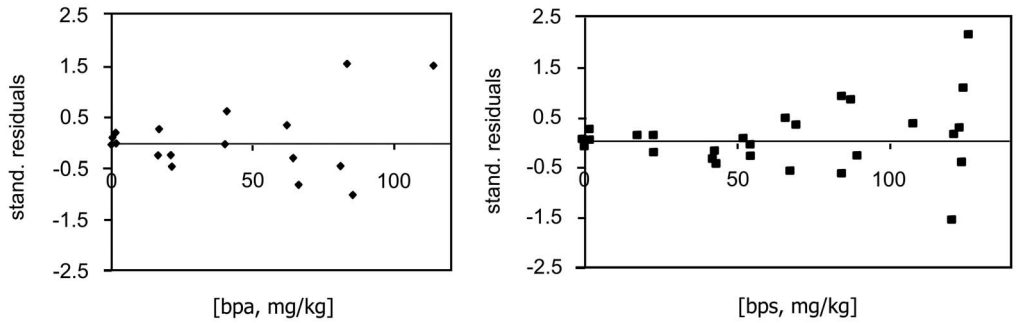


**Fig. 5** Standardized residual plot.

predicted values, divided by the square root of the residuals. In case of linearity, the standardized residuals are randomly distributed over the whole range and do not correlate with the concentration of the analyte. The random distribution validates the use of a linear model for the quantification between 0 and 100 mg $kg^{-1}$. In Fig. 5, the heteroscedasticity of the residuals can also be considered for the calibration model as an indication to apply a weighted regression model.[57] For the least square regression, constant variance across the concentration is assumed (homoscedasticity); otherwise, a weighted model should be used.

### 3.3. Calibration parameters and limit of detection

Calibration was carried out using the external calibration method. For each calibration curve, the parameters of the calibration and the confidence intervals were calculated according to Miller[57] and Edgerley[58] for a weighted regression because of the heteroscedasticity of the residuals. The variance of the signal rises proportionally to the concentration, therefore with the signal (Fig. 5). That is, the relative standard deviation (RSD) at single calibration levels is almost constant. The weighting factor is calculated from the standard deviation of the signal, as follows:[57]

$$w_i = \frac{s_i^{-2}}{\frac{1}{n}\sum s_i^{-2}}$$

$w_i$ = weighting factor; $s_i$= standard deviation; $n$ = number of values. The regression coefficients $a$ and $b$ in the model $y = bx + a$ were calculated as follows:[57]

$$b = \frac{\sum_i w_i x_i y_i - n\bar{x}_w \bar{y}_w}{\sum_i w_i x_i^2 - n(\bar{x}_w)^2}$$

$$a = \bar{y}_w - b\bar{x}_w$$

**Table 2** Parameters of the calibration curves for the quantification of bisphenol A and bisphenol S

| | 1 | 2 | 3 |
|---|---|---|---|
| *BPA* | | | |
| Slope | 307 ± 22 | 693 ± 44 | 181 ± 10 |
| Intercept | 2430 ± 145 | 1470 ± 190 | 329 ± 46 |
| Standard error | 56.2 | 18.9 | 3.67 |
| LOD/mg $kg^{-1}$ | 0.55 | 0.35 | 0.61 |
| LOQ/mg $kg^{-1}$ | 1.83 | 1.17 | 0.99 |
| Observations | 6 | 13 | 9 |
| *BPS* | | | |
| Slope | 256 ± 12 | 292 ± 10 | 117 ± 6 |
| Intercept | 702 ± 91 | 960 ± 109 | 831 ± 99 |
| Standard error | 4.36 | 4.4 | 2.6 |
| LOD/mg $kg^{-1}$ | 0.41 | 0.47 | 0.97 |
| LOQ/mg $kg^{-1}$ | 1.38 | 1.56 | 1.29 |
| Observations | 8 | 12 | 16 |

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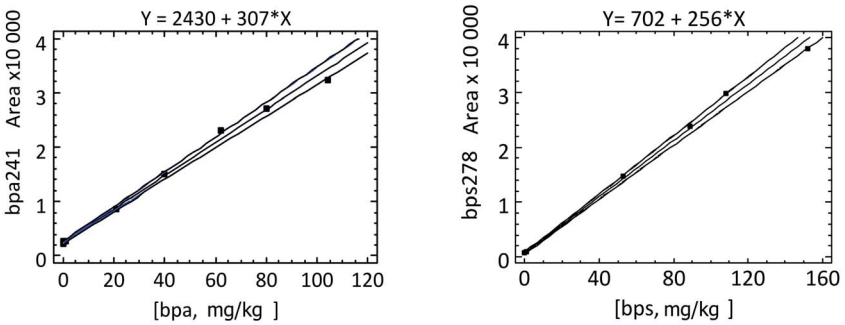


**Fig. 6** Plot fitted model for bisphenol A and bisphenol S calibration curve 1. Confidence intervals were calculated according to Edgerley.[58]

For the determination of the variance of the signal, which depends on the concentrations of bisphenol A and bisphenol S, a separate study was conducted on five samples for each calibration level. The relative standard deviations of the signal estimated were about 5% over the whole concentration range (data not shown).

The limit of detection and the limit of quantification were both calculated according to the regression approach, *i.e.* the method described by Mocak *et al.* 1997.[59] The limit of detection was calculated considering three times the standard error of the intercept. The limit of quantification was calculated considering ten times the standard error of the intercept of the regression (Table 2 and Fig. 6).

### 3.4. Precision and accuracy

As described in Section 2.1, different paper grades were analysed. The signals of bisphenol A, bisphenol S, and their neighbouring peaks do not overlap in the chromatograms from the paper sample analysis except for negligible background peaks. This enables the quantification of both analytes in paper, even in recycled paper. In addition, the ratio between both mass fragments of each compound was similar to the one calculated from the mass spectra of the pure analytes (Table 1 row 6). For the intra- and inter-assay validation of the method, samples for quantification were taken from different spots of the original sample in order to cover a wide area of the original sample. Each original sample was measured in triplicate within the measuring

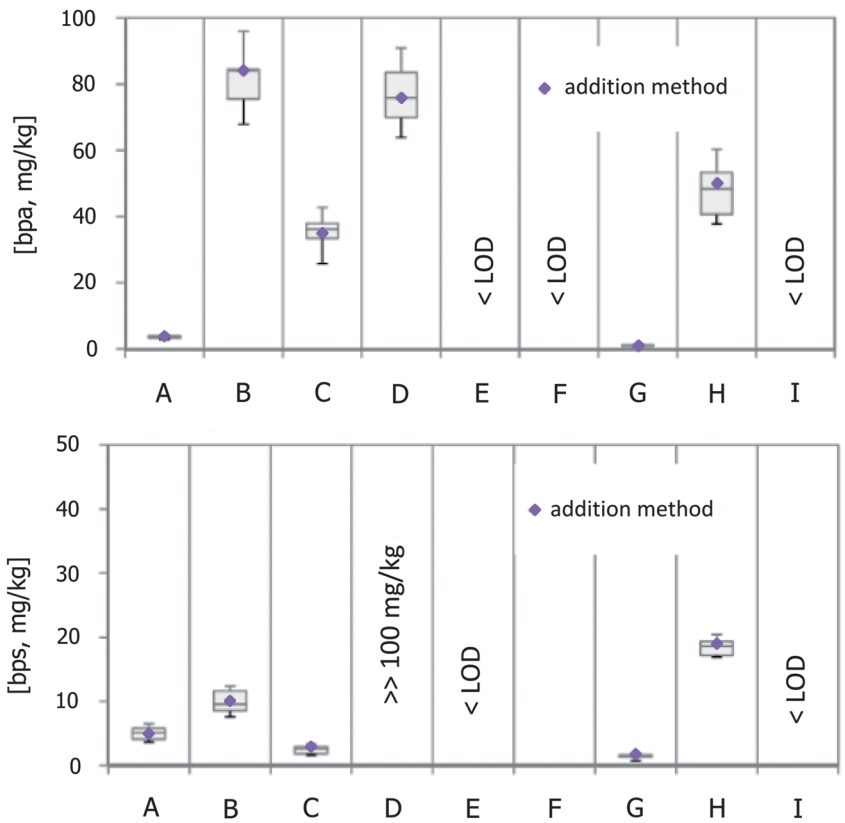


**Fig. 7** Box plot of the quantification results of BPA-free thermal paper samples (A–F); recycled paper (G–H) and virgin paper (I).

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**Table 3** Quantification results for paper samples obtained with the pyrolysis method and the extraction method

| Pyrolysis BPA/mg kg$^{-1}$ | Extracted BPS/mg kg$^{-1}$ |
|---|---|
| 1.3 | 2.5 |
| 2.4 | 2.6 |
| 4 | 3.1 |
| 17 | 17 |
| 70 | 60 |
| <1 | <1 |
| <1 | <1 |
| <1 | <1 |

sequence. The sequences were carried out in a time span of two months covering the influence of longer time ranges (Fig. 7). Concerning the paper samples investigated in Fig. 7, all samples containing bisphenol A contain bisphenol S as well. In the samples E, F, and I, the amount of bisphenol A or bisphenol S is lower than the limit of detection. In the case of sample D described as a BPA free paper, the amount of bisphenol S is much higher than in the other samples. It is assumed that in this sample bisphenol S was added as a substitute for the production of thermal paper instead of bisphenol A. The other samples contain low amounts of bisphenol A and bisphenol S. These amounts can be considered as "impurities" because the concentration of BPA in thermal paper containing bisphenol A as an additive has amounts of bisphenol A ranging from 0.3 to 1.7% (3000 to 17 000 mg kg$^{-1}$).[36]

Higher variations in the results are obtained in samples with higher analyte concentrations like samples B and D, in the case of bisphenol A. For samples with lower analyte concentration (A and G, in BPA and C and G in BPS), the variation is lower. This indicates that the method is more precise for the quantification of bisphenol A and bisphenol S in lower concentration ranges. On the other hand, the heterogeneity of our samples must be considered. The samples analysed, paper samples or solid samples in general, are less homogeneous than liquid samples. Results may vary because of the sample heterogeneity.

The accuracy of the method was confirmed with the standard addition method. Quantification with an external calibration curve can deliver inaccurate results because of a possible matrix effect. Such effects may change the signal obtained during the analysis. These matrix effects can be overcome by quantifying the samples with the standard addition method. The calculated concentrations of the analyte correspond very well to the results obtained when the external standard method is applied (Fig. 7). The accuracy of the method was also confirmed by evaluating some samples from a commercially independent laboratory with a conventional validated method, which includes analyte extraction. Sample pieces of 5 × 5 cm$^{-2}$ each were cut. The samples were refluxed with *n*-hexane–acetone and carbonate solution and the extract was derivatised. Substance group-specific fractioning was carried out with silica gel, and finally measured *via* GC/FID and GC/MS. Calibration and content determination were carried out *via* an external standard. Samples of the same production were measured in our laboratory. Table 3 shows the comparison.

The results obtained with the extraction method in the independent laboratory are close to the results obtained with the pyrolysis method, regardless of the sample complexity.

### 3.5. Internal standard

The criteria for the selection of an internal standard are given by the analyte properties and the system used. For the case of bisphenol A and bisphenol S, the internal standard chosen should be thermally desorbed from the matrix at a pyrolysis temperature of 500 °C without any breakdown of the molecule, or, better still, the behaviour within the analysis procedure should be the same as the analytes behaviour. In case of a derivatisation step in the analysis, the internal standard should also react in the same way as the analyte. In some cases, the compounds that best fit these criteria are the deuterized form of the analytes. The disadvantages are the costs and availability of the deuterized derivatives. The practicability of using an internal standard for the quantification of bisphenol A and bisphenol S with this technique was examined with the compounds diphenylmethane (DPM) and dibenzylether (DBE) but without success. As both compounds do not contain any hydroxyl groups, a derivatisation reaction with TMAH is not expected. Variations of the results obtained through deviations of standard methylation conditions will not be compensated for by using either DPM or DBE. However, deviations caused by other sources could be improved. In our study we did not get improved results by using these compounds as an internal standard. In both cases, the standard deviation of the signal within a sequence was not significantly increased. An improvement of the inter-assay reproducibility of the results was not achieved. In the case

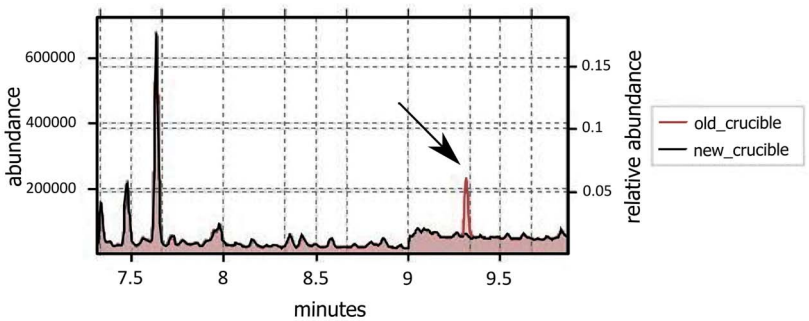


**Fig. 8** Identification peak for dibenzylether, *ca.* 50 mg kg$^{-1}$, in spruce paper, analysed in old and new crucibles.

of diphenylmethane, the analyte disappears with the time. This compound was not found to be as stable as bisphenol A or bisphenol S. In the case of dibenzylether, interesting results were observed confirming the impact of the crucible quality on the pyrolysis results, which are the reason to include the preliminary measurements with internal standards into the present publication. Quantification analysis of samples including both analytes, the derivatisation agent TMAH, and the compound DBE brings different results depending on the crucibles used. With old crucibles, DBE can be identified, but with new crucibles, the identification signal for DBE also disappears (Fig. 8). Contrary to the impact of crucible quality on signal response of BPA or BPS (see Section 3.1.2), a new crucible affects the signal response of DBE in such a way that DBE is not detected.

The reliability of the quantification using the method of an internal standard should not be ruled out for the analysis of traces with the analytical pyrolysis gas chromatography technique. More investigation in this field is needed. The utilization of deuterized standards as an internal standard should be taken in account.

## 4. Conclusions

An analytical method for the quantification of bisphenol A and bisphenol S in paper samples with a simplified preparation of samples has been developed. No extraction is needed. Low amounts of paper sample—about 120 μg—are sufficient for the quantification of trace amounts. In spite of the avoidance of any enrichment pre-treatment, sufficient low limits of determination below 1 mg $kg^{-1}$ for bisphenol A and bisphenol S in paper samples can be achieved. TMAH as a derivatisation agent was suitable for the on-line derivatisation reaction with bisphenols A and S without pre-treatment. The accuracy and the precision of the method (key characteristics for validation) suffice to quantify BPA and BPS in paper samples. The application of the quantification method can be extended to other materials, *i.e.* substrates. The reliability of quantification with an internal standard should be further investigated.

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